Antiproliferative Effect of Cucurbitacin B Extracted from *Trichosanthes cucumerina* L. on Human Cancer Cell Lines

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ABSTRACT

Objective: To determine the antiproliferative effect of cucurbitacin B extracted from *Trichosanthes cucumerina* L. on human cancer cell lines.

Methods: Two human lung non-small cell (adenocarcinoma) cancer cell lines i.e., LK87, and QG95, two human colon adenocarcinoma cell lines i.e., HCT15, and HT29, including one renal cancer cell line, A498, and one pancreatic cancer cell line, NOR-P, were used in this study. The viability of cells was assessed by using WST-8 which is based on detection of LDH released from damaged cells and reacts with WST-8 to form a yellow color. Cells were treated with the compound at various concentration from 1 through 100 μg/ml.

Results: The ED50 values (effective doses that are required for 50% inhibition growth of tumor cells) of the compound on human cancer cell lines ranged from approximately 69 μg/ml in HCT15 cells up to 231 μg/ml in QG95 cells. The inhibition of proliferation of this compound on these human cancer cell lines was observed to be in a dose dependent manner.

Conclusion: It could be concluded from this observation that this compound has a modest direct toxic effect to these cell lines with the highest toxic effect on human colon cancer cells.

Keywords: *Trichosanthes cucumerina*, Buap-Khom, cucurbitacin B, human cancer cell lines

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Trichosanthes cucumerina L., named in Thai "Buap- Khom", is a plant in the Cucurbitaceae family which is commonly found in Southeast Asia and Australia. From several reports, plants in this family are composed of many genera accounting for 110 genera and about 640 species which are mostly woody or herbaceous with climbing or trailing stems bearing tendrils and often arising from a tuberous rootstock.[1,2] In Thailand, this plant wildly grows along the river in some provinces such as Kanjanaburi. For Thai folk medicine, some of them have been used for the properties of antihelmintic, antidiabetic, and antiinflammatory effects except for *Trichosanthes cucumerina* which is inedible because of the bitter taste inducing nausea and vomiting symptoms.[3] In addition, in other countries, such as India, these special group of plants especially their seeds and fruit have been prescribed to treat various diseases i.e., infections and malignancies.[4] Several investigations have demonstrated the striking cytotoxic activities of these familial plants isolated compounds, cucurbitacins which are the major component, against several human cancer cell lines such as breast and lung cancer cells.[5-7]

In this study, cucurbitacin B was isolated from the juice of the fruit of *Trichosanthes cucumerina* and purified. This compound was tested against several human lung, colon, pancreatic, and renal cancer cell lines which have not previously been investigated.

MATERIALS AND METHODS

Plant extraction process

30 kg of fresh fruit of *Trichosanthes cucumerina* were collected from Kanjanaburi Province. They were immediately pressed and filtered to get the juice. After

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getting the juice (5 liters), it was shaken in ether in the separatory funnel and 1 gram of crystalline mixture was obtained. The crystalline mixture was purified by reversed phase column (RP-18) with the solvent system of water and acetonitril (55:45).[8] The major component which comprises cucurbitacin B (mixtured with dihydrocucurbitacin B) was gained from this extraction and purification procedure.

Extract preparation

For preparation, 0.0015 gm of the compound was dissolved in 50 µl of 95% ethanol and added up with 950 µl media to get the 1 ml stock. For all the experiment, the stock was diluted with media to get the concentrations at 10, 50, 100, 500, and 1,000 µg/ml. For the final concentrations from 1-100 µg/ml, 10 µl of each prepared diluted compound was added to each well in 96 well plates.

Cell lines and culture

Two human lung non-small cell (adenocarcinoma) cancer cell lines i.e., LK87, and QG95, two human colon adenocarcinoma cell lines i.e., HCT15, and HT29, one renal cancer cell line, A498, and one pancreatic cancer cell line, NOR-P, were used in this study. Human lung cancer cell lines were kindly provided by Dr.Koichi Takayama and Dr.Hiroyuki Inoue, Research Institute of Diseases of the Chest, Kyushu University, Japan. The other cell lines were kindly provided by Dr.Kenzaburo Tani, Medical Institute of Bioregulation, Kyushu University, Japan. All human lung and colon cancer cells were maintained in RPMI1640 (Nacalai Tesque, Kyoto, Japan) except for HT29 colon cancer cell line which was maintained in McCoy's 5A (Gibco, USA). Human renal carcinoma cell line was maintained in MEMalpha (Gibco, USA) and pancreatic cancer cell line was maintained in DMEM (Nacalai Tesque, Kyoto, Japan). All medium were supplemented with 10% fetal bovine serum (Japan Bioserum, Japan) and 1% antibiotic plus antimycotic agent (Nacalai Tesque, Kyoto, Japan). All cell lines were incubated at 37°C with 5% CO_2 and humidified atmosphere.

Cell viability assay

Viability of cells was assessed by using WST-8 (Nacalai Tesque, Kyoto, Japan) asssay (2-2(methoxy-4-nitrophenyl)3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt), which is based on detection of LDH releasing from damaged cells and reacts with WST-8 to form a yellow color.[9] In brief, cells were seeded at a density of $1x10^4$ cells/well in 96 well plates and treated with the compound at various concentrations from 1 through 100 µg/ml. Doxorubicin was used as a positive control. After a 48-hour incubation, 10 µl of WST-8 was added to each well and incubated at 37°C with a 5% CO_2 incubator for an additional 4 hours. The absorbance at 450 nm. of the dissolved solution was measured by using an Elisa plate reader (ThermoLabsystem, Japan). Data was calculated by using the formula as followed[10]:

Cell death (%) = [(control O.D.-sample O.D.)/ control O.D.] x100

Cell viability = 100-(% Cell death)

Statistic analysis

All experiments were performed in triplicate with three experiments. Data were expressed as the mean ± standard deviation. The R square equation was used to calculate the ED50 value. A P-value less than 0.05 were considered statistically significant.

RESULTS

Antiproliferative effects of compound on human cancer cell lines.

The ED50 values (effective doses that are required for 50% inhibition growth of tumor cells) of the compound on human cancer cell lines were summarized in Table 1 ranging from approximately 69 µg/ml in HCT15 cells up to 231 µg/ml in QG95 cells.

The antiproliferative effect, expressed as cell viability, of colon cancer cell lines, HCT15 and HT29, renal cancer cell, A498, and pancreatic cancer cell, NOR-P, showed the modest inhibition by the compound with the ED50 values at 69.391 ± 18.382, 106.431 ± 20.756, 105.912 ± 3.057, and 87.396 ± 1.950 µg/ml respectively as shown in Fig. 1A and 1C. The compound inhi-bited growth of lung cancer cells, LK87 and QG95, in the modest to low activity with the ED50 values at 99.517 ± 6.116 and 231.830 ± 11.182 µg/ml respectively as illu strated in Fig. 1B. All inhibition of cancer cells by the compound on the proliferation of these human cancer cell lines was observed to be in a dose dependent manner.

DISCUSSION

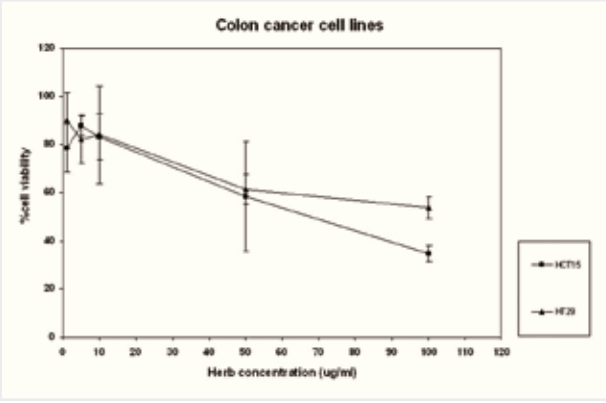
This investigation of the antiproliferative effects of cucurbitacin B was performed on several human cancer cell lines which have never been previously reported. The biological inhibitory effect of the compound depends on not only healthy or malignant cells but also the difference in the type of cell lines. Comparing in all these cancer cells, this compound has a modest to low activity on human lung cancer cell lines, whereas the inhibitory activity of the compound on other cell lines was in a modest pattern. Therefore, it could be concluded from this observation that this compound has a modest direct toxic effect to these cell lines.

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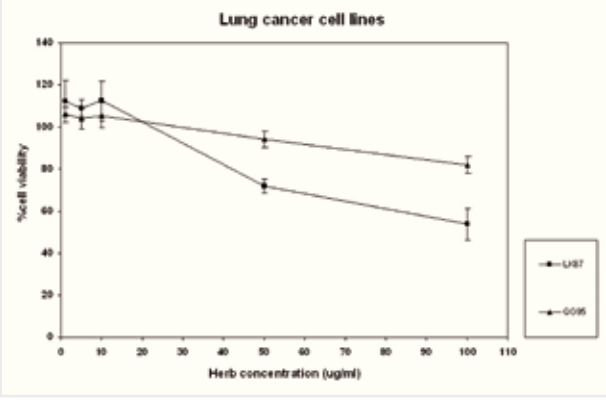
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TABLE 1. ED50 values of human cancer cell lines were demonstrated as mean ± standard deviation.

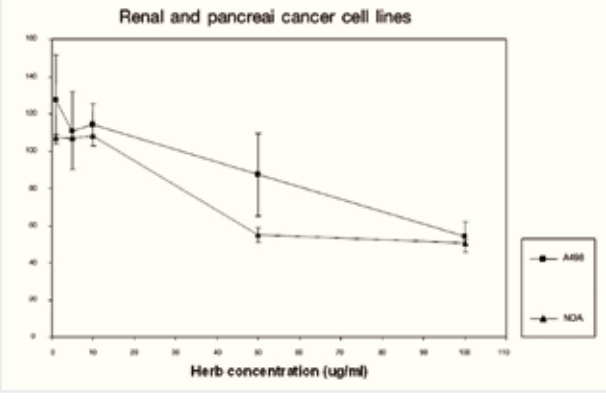
Type of cancer cell line	ED50 value (mean SD)
Colon cancer: HCT15	69.391 ± 18.382 µg/ml
HT29	106.431 ± 20.756 µg/ml
Renal cancer: A498	105.912 ± 3.057 µg/ml
Pancreatic cancer: NOR-P	87.396 ± 1.950 µg/ml
Lung cancer: LK87	99.517 ± 6.116 µg/ml
QG95	231.830 ± 11.182 µg/ml



1A



1B



1C

Fig 1. Proliferative inhibition of cucurbitacin B on two colon cancer cell lines, two lung cancer cell lines, one renal cancer cell line and one pancreatic cancer cell line. The cells were seeded at $1x10^4$ cell/well in 96 well plates for 48 hours. HCT15 and HT29 colon cancer cell lines (1A), LK87 and QG95 lung cancer cell lines (1B) A498 renal cancer cell line and NOR-P pancreatic cancer cell line (1C) at different concentrations of 1, 5, 10, 50, 100 and 150 µg/ml for 48 hours. The cell viabilities were determined by MTT assay.

REFERENCES

1. Benson L. Plant Classification 1st ed. United States of America: D.C. Health and Company; 1959.p.287-9.
2. Jeffrey C. Flora of Tropical East Africa, The Whitefriars Press Ltd., London and Tonbridge; 1967.p.1.
3. Silapa-archa W. Isolierung und strukturaufklarung chemischer komponenten aus Trichosanthes cucumerina L. Bonn: Rheinischen Friedrich-Wilhelms-Universitat; 1987.
4. Liu T, Zhang M, Zhang H, Sun C, Deng Y. Inhibitory effects of cucurbitacin B on laryngeal squamous cell carcinoma. Eur Arch Otorhinolaryngol. 2008 Oct;265(10):1225-32.
5. Tannin-Spitz T, Grossman S, Dovrat S, Gottlieb HE, Bergman M. Growth inhibitory activity of cucurbitacin glucosides isolated from Citrullus colocynthis on human breast cancer cells. Biochem Pharmocol. 2007 Jan 1;73 (1):56-67.
6. Clericuzio M, Tabasso S, Bianco MA, Pratesi G, Beretta G, Tinelli S, et al. Cucurbitane triterpenes from the fruiting bodies and cultivated mycelia of Leucopaxillus gentianeus. J Nat Prod. 2006 Dec;69(12):1796-9.
7. Clericuzio M, Mella M, Vita-finzi P, Zema M, Vidari G. Cucurbitane triterpenoids from Leucopaxillus gentianeus. J Nat Prod. 2004 Nov;67(11): 1823-8.
8. Jiratchariyakul W, Moongkarndi P, Theppeang K, Sethajintanin D, Jarikasem S, Frahm AW. Cytotoxic principles from *Trichosanthes Cucumerina* L. Thai J Phytopharm. 1999 Dec;6(2):1-9.
9. Brady AJ, Kearney P, Tunney MM. Comparative evaluation of 2,3-bis [2-methyloxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide (XTT) and 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt (WST-8) rapid colorimetric assays for antimicrobial susceptibility testing of staphylococci and ESBL-producing clinical isolates. J Microbiol Methods. 2007 Dec;71(3):305-11.
10. Hsu YL, Kuo PL, Chiang LC, Lin CC. Isoliquiritigenin inhibits the proliferation and induces the apoptosis of human non-small cell lung cancer A549 cells. Clin Exp Pharmacol Physiol. 2004 Jul;31(7):414-8.